THE DEVELOPMENT OF THE EMBRYO-SAC AND EMBRYO OF CLAYTONIA VIRGINICA.*

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Claytonia virginica Linn. was selected as a type for class study. The number of interesting points led me to complete the series of preparations and prepare the results for publication. The wide distribution and the ease with which the material can be killed and prepared for class work may make it an equally desirable type for others who may wish to study a dicotyl with unequally developed cotyledons.

METHODS.

The material was killed and fixed in Fleming's solution, passed through the alcohols, imbedded in paraffin and cut on a Minot microtome. In most cases the sections were cut rather thick. For the very young stages only the calyx was removed. The fluid will penetrate the ovules readily until they are old enough to change their color from white to brown or black; after that it is necessary to puncture the integuments. A combination of anilin safranin and gentian violet gave the best results.

MEGASPORES AND EMBRYO-SAC.

The single archesporial cell is hypodermal in origin, and can be easily recognized from the surrounding cells of the nucellus. From this a single tapetal cell is formed, which may divide again either by anticlinal or periclinal walls (Figs. 1, 2*b*). In a very few cases three tapetal cells were observed (Fig. 2*b*). Four megaspores are formed in the usual manner (Figs. 2*a*, 2*b*). The lower or functional megaspore enlarges at the expense of the three potential megaspores and the tapetal cells (Fig. 3). The functional megaspore now enlarges, giving rise to the two, four and eight celled embryo-sac in the usual manner (Figs. 4, 5, 6, 8). In the four-celled stage the nuclei are approximately equal in size. In the eight-celled stage the synergids are very large and pear-shaped, and at least one persists until a very late stage in the development of the embryo (Figs. 9, 10, 12, 13, 14, 16, 18). The egg is slightly larger than the synergids and very similar in appearance; while the polar nuclei are comparatively large (Figs. 6, 7). The antipodals are somewhat smaller and cut off from the sac by delicate but definite walls (Fig. 6), and always occupy about the same relative position to each other.

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The antipodals are absorbed very early (Fig. 8), and the sac enlarges very rapidly, especially from the antipodal end, forming almost a complete circle and enclosing a mass of the basal cells of the nucellus in the center of the camphylotropous ovule Fig. 22). The first division of the endosperm occurs at about the same time as the first division of the embryo. After that it divides very rapidly, forming the typical peripheral endosperm* lining the embryo-sac (Fig. 16). At the antipodal end of the sac the endosperm is always more dense than in other parts of the sac (Fig. 17), and probably makes the absorption of the nucellus more rapid. The micropyle and the pollen tube (Fig. 16) were very clear in many preparations, but the act of fertilization was not observed. However, several cases were observed which indicated that the second sperm nucleus might unite with the two polar nuclei, but were not sufficiently clear to draw a conclusion.

EMBRYO.

The fertilized egg divides by transverse walls to form three or four cells in an axial row (Figs. 9–12). Typically the row consists of three cells developed in acropetal order. The upper of these cells next divides by a longitudinal wall (Fig. 10). This is followed by a similar division in the next lower cell (Fig. 11). The two upper cells now divide by longitudinal walls at right angles to the first, thus forming a quadrant (Fig. 12). In the meantime one or more transverse walls have been formed in the basal cell, thus lengthening the suspensor. The embryo proper is now usually composed of three or four tiers of cells (Figs. 13, 14, 15). Each tier of cells divides first longitudinally and then more or less irregularly, by both transverse and longitudinal walls, forming an embryo almost spherical in shape, but slightly larger on the side away from the funiculus (Figs. 13, 14, 15, 16, 18). The protoplasm in the upper two-thirds of the embryo is usually more dense than in the lower one-third (Fig. 16).

The suspensor originates as a single cell (Figs. 9–12). This cell usually divides by the formation of transverse walls (Figs. 13, 14, 16), but occasionally divides by longitudinal walls (Fig. 15). The formation of transverse walls is followed by a longitudinal division in either one or both cells (Figs. 16, 18). After this it was impossible to follow the divisions. However, it always remains short, but becomes very much widened (Fig. 19) as a result of longitudinal division. By the time the embryo has reached three-fourths full size the suspensor has usually disappeared (Fig. 20).

The cotyledons originate from opposite points of the almost spherical embryo (Fig. 18, c, c). The outer of these two points

* Hegelmaier, Dr. Untersuchungen ueber die Morphologie der Dicotyledonen-Endosperms. Nova Acta d. K. L. C. D. Akad. d. Naturforcher 49. 1885.

grows very rapidly and gives rise to the very large cotyledon (Fig. 19). This cotyledon grows very rapidly and curves into almost a complete circle (Fig. 22). The inner point of growth makes very little increase in size and forms the inner rudimentary cotyledon, which now appears as a small projection almost at right angles to the large cotyledon (Figs. 19, 20, 21).

The plumule originally stands at the upper end of the axis of the embryo (Fig. 18), but with the development of the large cotyledon it is pushed to one side, so that in the older stages it appears as a lateral plumule (Figs. 19, 20, 21).

The development of the calyptrogen begins in the dermatogen and in the cells just above the suspensor (Fig. 19*a*), and gradually extends across the tip of the embryo. The root cap is formed in the usual manner, by transverse division of cells in this layer, and about this time the suspensor begins to disappear.

SUMMARY.

1. Normally four megaspores and two tapetal cells are formed, the lower megaspore cell forming the embryo sac in the usual manner.

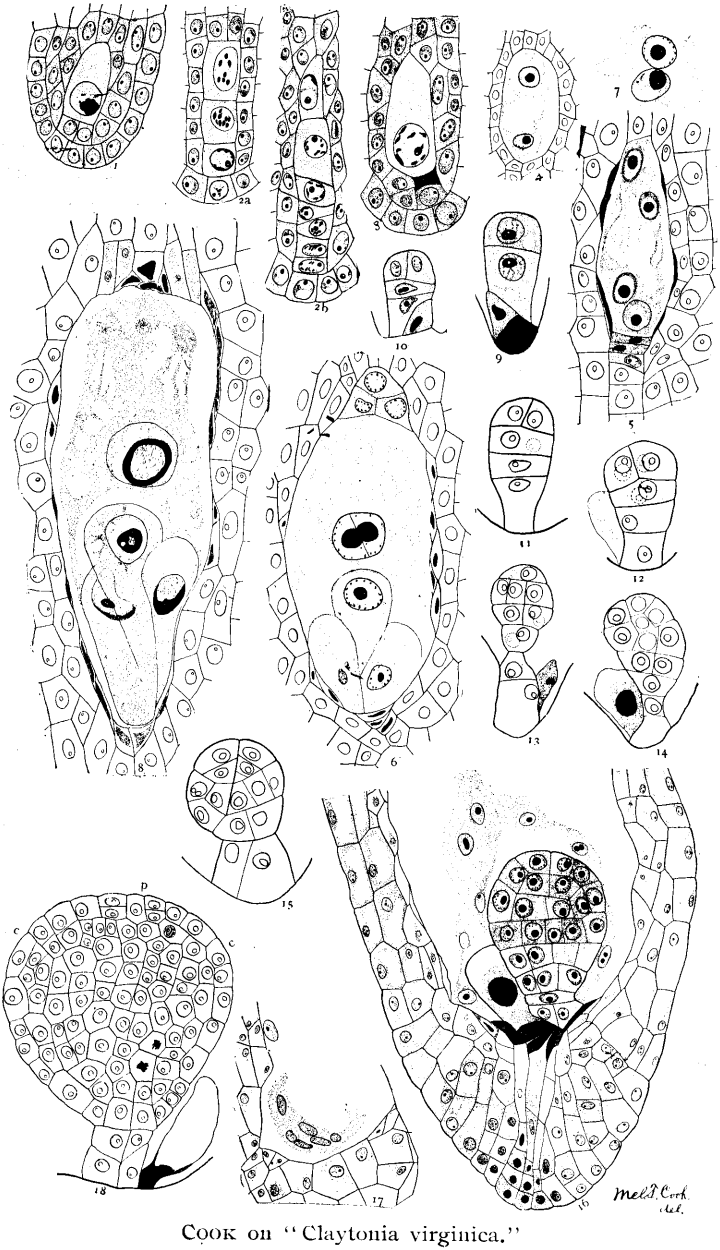
2. The first five or six divisions in the formation of the embryo are quite regular, but the succeeding divisions are very irregular.

3. The suspensor is at first filamentous, but becomes massive by longitudinal divisions. It does not contribute to the formation of the tissues of the root-tip.

4. Only one cotyledon develops and it becomes very large; the other cotyledon remains rudimentary and gives the mature embryo the appearance of a monocotyl.

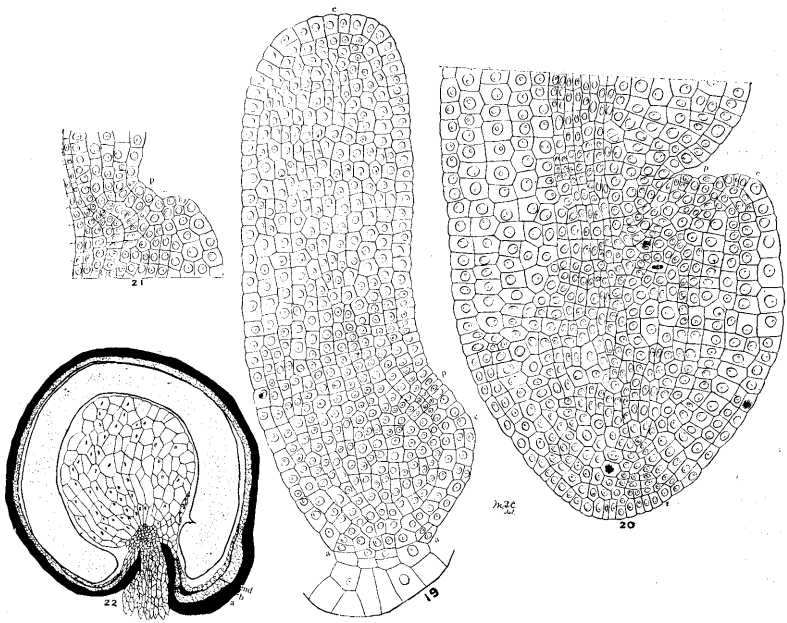
I wish to express my thanks to Professor J. H. Schaffner, of the Ohio State University, for valuable suggestions in the completion of this study. I also wish to express my thanks to three of my former students in DePauw University, Mr. J. W. Little, Miss Nellie Inwood and Miss Helen Powell, for valuable material and preparations.

OHIO NATURALIST. *Plate 5.*



COOK on "Claytonia virginica."

OHIO NATURALIST. *Plate 5a.*



COOK on "Claytonia virginica."

EXPLANATION OF FIGURES.

In the drawings, Leitz stand and a B. and L. camera lucida were used. Figs. 1 to 8, a No. 6 Zeiss ocular and a 1-12 B. and L. immersion; in Figs. 9 to 21, a No. 6 Zeiss ocular and a No. 7 Zeiss objective; in Fig. 22, a No. 4 Leitz ocular and a No. 5 Leitz objective.

Fig. 1. Archesporial cell and two tapetal cells.
" 2*a*. One tapetal cell and beginning of the second division in the formation of the megaspores.
" 2*b*. Four megaspores and three tapetal cells.
" 3. Functional megaspore.
" 4. Two-celled sac.
" 5. Four-celled sac and tapetal cells.
" 6. Eight-celled sac, showing conjugation of polar nuclei. Also the three antipodal cells just before disorganization.
" 7. Polar nuclei approaching.
" 8. Eight-celled sac after conjugation of polar nuclei. First stage in absorption of nucellus from antipodal end of sac.
" 9. Two-celled embryo and persistent synergid.
" 10. Four-celled embryo and persistent synergid.
" 11. Six-celled embryo.
" 12. Eight-celled embryo and peristent synergid.
" 13. Embryo " " "
" 14. " " " "
" 15. " with longitudinal wall in first suspensor cell.
" 16. " and persistent synergid. Also endosperm.
" 17. Antipodal end of sac, showing massing of endosperm (about same age as in Fig. 16).
" 18. Spherical embryo and persistent synergid: c, cotyledon; p, plumule.
" 19. Embryo showing suspensor; c, cotyledons (one large and one small); p, plumule; and formation of calyptrogen (a) above suspensor.
" 20. Base of large embryo showing plumule (p), rudimentary cotyledon (c), and root-cap (r).
" 21. Part of embryo showing rudimentary cotyledon (c) and plumule (p).
" 22. Entire ovule, showing mature embryo, with large cotyledon curved around a central mass of nucellus cells, which are rich in starch; a, outer integument; b, inner integument; end., endosperm.